Analysis of Gravity-Induced Particle Motion and Fluid Perfusion Flow in the NASA-Designed Rotating Zero-Head-Space Tissue Culture Vessel

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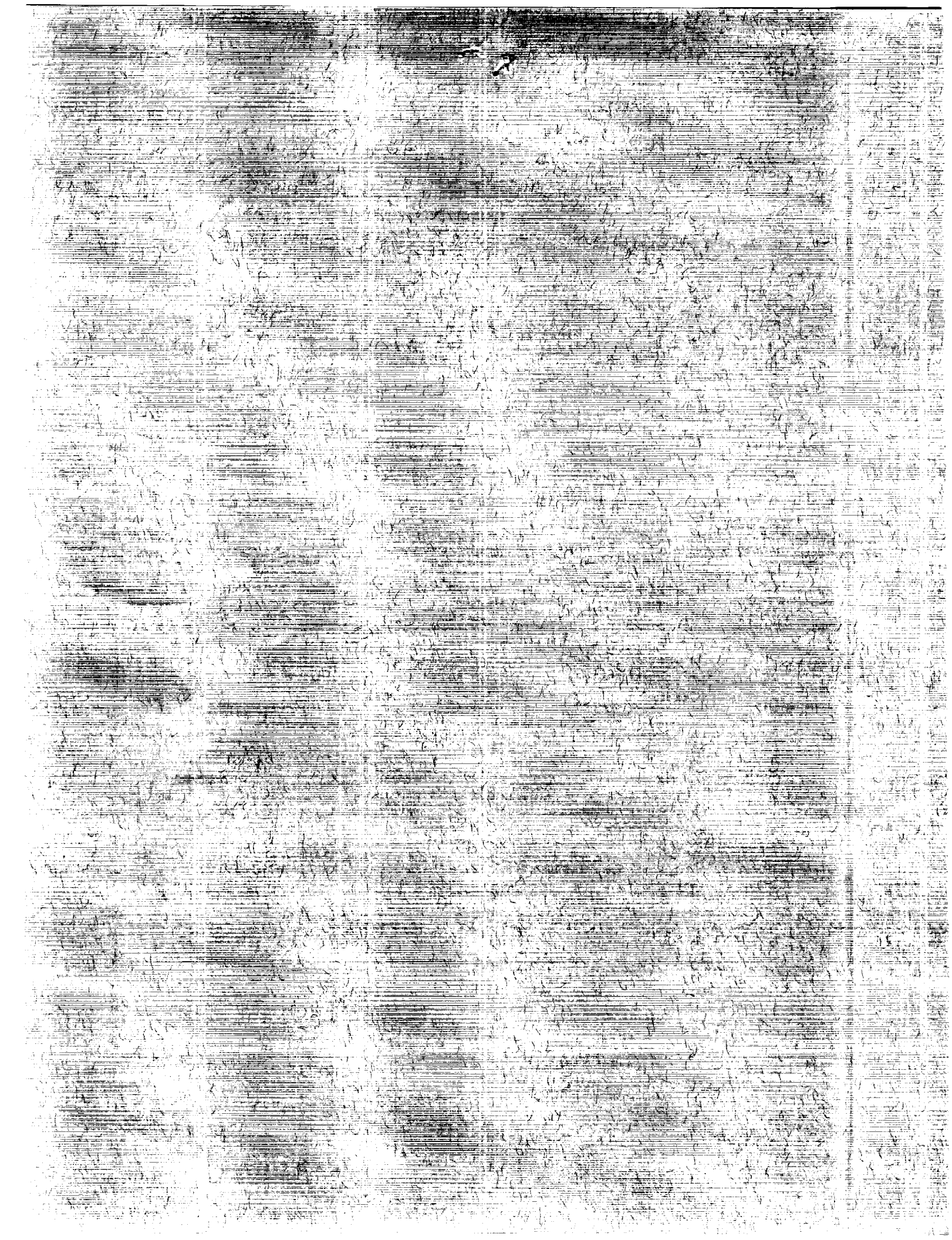**NASA**

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CONTENTS

I. Introduction and Approach

It is the purpose of this analysis to quantitatively calculate the gravity-induced motions, through the culture media, of living tissue segments cultured in the NASA rotating zero-head-space culture vessels. This is then compared with the media perfusion speed which is independent of gravity. The results may be interpreted as a change in the physical environment which will occur by operating the NASA tissue culture systems in actual microgravity (versus unit gravity).

The equations governing particle motions which induce flows at the surface of tissues contain "g" terms. This allows calculation of the fluid flow speed, with respect to a cultured particle, as a function of the external gravitational field strength. The analysis is approached from a "flow field" perspective. Flow is proportional to the shear exerted on a structure which maintains position within the field. On the average (over time and space) the cultured particles must maintain their position within the fluid flow field in order to remain dispersed and within the boundary of the culture vessel walls.

The equations are solved for the deviation of a particle from its original position in a circular streamline as a function of time. Polar coordinates are utilized and the equations are solved for the radial deviation, $Dr(t)$, and the tangential deviation, $Dt(t)$, as a function of time. The radial deviation is particularly important for defining the operating limits and dimensions of the vessel because of the finite radius at which particles necessarily intercept the wall.

This analysis utilizes a rotating reference frame concept. This rotating reference frame is stationary with respect to the rotating culture vessel and fluid medium. Coriolus and centrifugal pseudo forces are apparent in this reference frame because it is circularly accelerated.

II. Rationale

High order morphologic structures which compose the surface and interior of cultured tissue are assumed to experience shear in proportion to the speed of flowing fluid across their surfaces. The absolute magnitude of this induced shear is dependent on the details of the particular tissue architecture and properties of the shearing fluid. Other biologic phenomena such as cell division mechanics, cell interaction residence time, ligand to receptor interactions, cell to anchorage substrate attachment (and separation) dynamics, membrane associated stress transduction, and the orientation of multiple cell types with extracellular matrix in coculture are expected to be directly responsive to this surface flow phenomenon. Such biomechanical phenomena at the molecular level may behave in a highly nonlinear fashion and theoretical models are only beginning to emerge. Therefore, this analysis does not predict the biological response of cultured tissue to the calculated surface flows, but addresses the physical character of the culture environment.

III. Mechanisms Leading to Surface Flows

We quantify three primary component mechanisms which induce surface flows across cultured tissues in the rotating perfused bioreactor vessel.

1. Gravity-induced distortion of tissue paths from fluid streamlines.

2. Centrifugal force and coriolus-induced particle motion.

3. Perfusion of fluid medium through the culture vessel.

The first two mechanisms are the result of vessel rotation in a gravitational field and will be effectively eliminated in microgravity. They both are continuously present in unit gravity. The third mechanism is independent of gravity and rotation. The time profile may be controlled by intermittent perfusion of the culture

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vessel. The three mechanisms above are quantified in the following analyses. The results are expressed in units of centimeters/second and represent the calculated fluid flow speed at the surface of a typical segment of cultured tissue.

IV. Gravity and Rotationally Induced Distortion of Particle Path from Streamline

Figure 1 shows a particle at position P within a fluid medium rotating at angular rate ω. It will be shown that the actual path of the particle is nearly the path (dotted line) which is displaced to the left of the circle with radius Ro. The angle, α, is defined by the position vectors Ro and P. At time $t = 0$ the particle is at position Po with radius Ro. Over the differential time period, dt, the particle defines the differential angle, dα. The gravity vector, g, always points down as observed from the nonrotating reference frame.
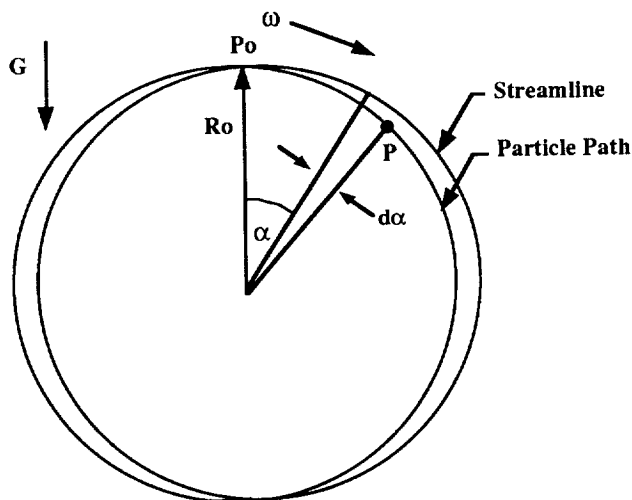

Figure 1. Particle in a Horizontally Rotating Field

Figure 2 shows the region of P. The individual velocity vectors in the rotating (circularly accelerated) reference frame which contribute to the resultant particle motion through the medium are listed below. These same velocity vectors may be derived from the forces acting on a particle under the influence of a rotating viscous fluid.

Radial acting components:

Vcr - outwardly directed particle velocity due to centrifugal force

Vsr - radially inward directed component of gravity-induced sedimentation

Tangential acting components:

Vst - tangential-directed component of gravity-induced sedimentation

Vct - tangential coriolus-induced motion due to rate of change of radius (of particle)
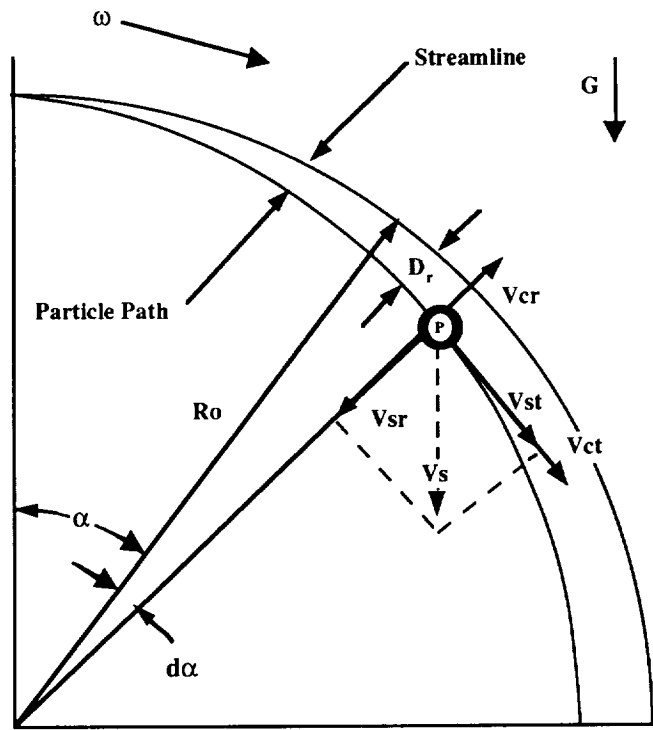
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Figure 2. Vector Velocity Diagram

Accelerations may be related to particle "terminal' velocities by the Stokes equation (1). The terminal velocity obtained by a particle falling in a fluid medium is directly proportional to the acceleration due to gravity. Because a particle cannot distinguish between the acceleration of gravity and acceleration due to reference frame, we are able to apply this result to calculate the terminal velocity obtained by a particle within an accelerated reference frame.

$$Vs = \frac{2\Delta d\, g r^2}{9v}$$

Vs = Stokes terminal sedimentation rate
Δd = density difference
r = particle radius
v = viscosity
g = acceleration of gravity

(1)

As the fluid rotates at angular rate ω, the acceleration due to gravity (gravity vector) in the rotating reference frame is observed to rotate at this same angular rate. The gravity-induced particle motion vector, Vs, is shown as a dotted line because it is resolved into component radial and tangential vectors Vsr and Vst for analysis. We will now show that for tissue particles of current and anticipated interest the terminal sedimentation rate (or Stokes velocity) is reached in such a short period of time that the change in direction of the gravity vector is negligible. Under these conditions the gravity-induced component of particle motion will be at well over 99% terminal velocity and in the direction of the gravity vector.

Our laboratories have measured the typical terminal velocity of cultured tissues to be 0.5 centimeters/second in unit gravity. Larger pieces fall faster and smaller pieces tend to fall slower. The results of this analysis are scaled to Vs = 0.5 centimeters/second.

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Time to Reach Terminal Velocity Calculation

The force, Fr, resisting the motion of a particle travelling at velocity Vp through a fluid medium may be expressed as equation (2). Because the force is oppositely directed from the motion, k is a negative constant.

$$Fr = -k\,Vp$$

(2)

Application of Newton's second law derives equation (3).

$$mg - k\,Vp = ma$$

(3)

At terminal velocity Vt the acceleration, a, of the particle is zero because the resistance force exactly balances the force on a mass, m, under the acceleration of gravity, g. Letting $a = 0$ and solving for Vt gives equation (4). We measure Vt to be 0.5 centimeters/second.

$$Vt = \frac{mg}{k} = 0.5\ cm/\text{sec}$$

(4)

Equation (3) may be solved for $Vp(t)$ to give equation (5). This shows that the particle speed will exponentially approach terminal velocity with a time constant of m/k seconds.

$$Vp = Vt(1 - e^{\frac{-kt}{m}})$$

(5)

Equation (4) may be solved for the time constant $\tau = m/k$. Substituting the measured value for Vt gives the time constant equation (6).

$$\tau = \frac{m}{k} = \frac{Vt}{g} = \frac{0.5\ cm/sec}{980\ cm/sec^2} = 5.1 \times 10^{-4}\ sec$$

(6)

The particle will reach over 98% of its terminal velocity in approximately 2 milliseconds. At the maximum RPM used in this analysis (100 RPM), this corresponds to an angle, α, of less than 1.3 degrees. Therefore, the gravity vector direction changes by less than 1.3 degrees (at maximum RPM) in the time required for the particle to attain over 98% of its terminal velocity. For all practical purposes, the tissue segments are travelling through the culture media at their terminal Stokes velocity vectorially added to the centrifugal- and coriolus-induced velocities. We will find that the particles travel in small, nearly circular paths (distorted by centrifugal and coriolus effects) in the rotating reference frame at the same angular rate as the vessel rotation.

From Figure 2 we may now write equations (7) and (8) for the radial and tangential particle velocities as observed in the rotating reference frame. In this reference frame the circularly rotating fluid is stationary and accelerations due to the pseudo forces, centrifugal and coriolus, appear. Standard vector operations are valid in this reference frame so long as the pseudo forces are considered.

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$$Vr = \text{Radial particle velocity} = Vcr - Vsr$$

$$\text{(7)}$$

$$Vt = \text{Tangential particle velocity} = Vst + Vct \tag{8}$$

The expression for the radial acceleration of a particle travelling on a circular path is well known as equation (9).

$$Ar = \omega^2 r \tag{9}$$

The outward-directed radial velocity of the particle at position P, due to this acceleration, may be written in terms of the Stokes terminal velocity as equation (10). G is a constant, 980 centimeters per second squared, and does not vary with the external gravitational field. The effect of differing external gravitational fields is determined by altering Vs, which is proportional to the external gravitational field. The function $Dr(t)$ is the radial deviation of the particle from the ideal circular streamline as a function of time (see figure 2).

$$Vcr = Vsg\ \frac{\omega^2[Ro + Dr(t)]}{G} \qquad \begin{array}{l} G = 980\ \text{cm/sec}^2 \\ Vsg = \text{Stokes terminal velocity} \\ \qquad\quad \text{(unit gravity)} \end{array}$$

$$\text{(10)}$$

The gravity-induced radial velocity component, Vsr, may be written as equation (11). This component varies cosinusoidally as the gravity vector rotates in the rotating reference frame (or as the vessel rotates in the fixed reference frame).

$$Vsr = Vs\cos(\omega t)$$

$$\text{(11)}$$

The gravity-induced tangential velocity component, Vst, may be written as equation (12). This component varies sinusoidally as the gravity vector rotates in the rotating reference frame (or as the vessel rotates in the fixed reference frame).

$$Vst = Vs\sin(\omega t)$$

$$\text{(12)}$$

The coriolus-induced tangential velocity arises because as the particle moves inward or outward radially it is exposed to streamlines with slower or faster tangential speeds, respectively. The resulting tangential acceleration, which is dependent on the rate of this radial motion, was defined as Vr in equation (7). The coriolus-induced tangential acceleration, Ac, due to the rate of change in radius dr/dt for a fixed angular rotation rate ω, is well known as equation (13).

$$Ac = -2\omega\ \frac{dr}{dt}$$

$$\text{(13)}$$

The tangential motion of the particle due to the coriolus acceleration may be written in terms of Vs, G, ω, and Vr as equation (14). G is used in this case (instead of g) as the constant, 980 centimeters per second squared, to avoid confusion with the external gravitational field strength which does not contribute to this component of particle motion. Vsg is a numerical constant equal to the unit gravity Stokes sedimentation velocity.

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$$Vct = -Vsg \frac{2\omega}{G} Vr$$

(14)

The results of equations (10), (11), (12), and (14) may be substituted into equations (7) and (8) to produce the following vector equations, (15) and (16), describing the particle radial and tangential velocities. These equations are valid for all time $0 < t <$ infinity.

$$Vr = Vcr - Vsr = \frac{Vsg \, \omega^2 \, [Ro + Dr(t)]}{G} - Vs \cos(\omega t)$$

(15)

$$Vt = Vct + Vst = Vs \sin(\omega t) - Vsg \frac{2\omega}{G} Vr$$

(16)

Equations (15) and (16) may be integrated (over time) to obtain the particle deviations in the radial (across streamlines) and tangential (along streamlines) directions. The radial deviation is particularly important because it defines the particle orbit radius and, therefore, the conditions over which the rotating vessel performs well.

Radial Deviation Calculation

Equation (15) is integrated as follows to derive the radial deviation of the particle from the original circular streamline at radius Ro.

$$\int_0^t Vr(t)dt = Dr(t) = Vsg \int_0^t \frac{\omega^2 Ro}{G} dt + Vsg \int_0^t \frac{\omega^2 Dr(t)}{G} dt - Vs \int_0^t \cos(\omega t)dt$$

(17)

$$Dr(t) = Vsg \left| \begin{array}{c} t \\ \\ 0 \end{array} \frac{\omega^2 Ro \, t}{G} - \left| \begin{array}{c} t \\ \\ 0 \end{array} \frac{Vs}{\omega} \sin(\omega t) + Vsg \int_0^t \frac{\omega^2}{G} Dr(t) \, dt$$

(18)

The term which integrates $Dr(t)$ may be physically interpreted as the accumulation of centrifugually induced, outwardly directed deviation from previous vessel revolutions. For a single vessel rotation with a particle beginning at radius Ro this term may be dropped and the equation is extremely accurate for $0 < t < 2\pi/\omega$. Equation (19) becomes the solution for the radial-directed deviation from the circular streamline for a particle beginning at radius Ro, as in figure 1. This equation is constrained to a single revolution with the particle beginning at radius Ro at $\alpha = 0$.

$$Dr(t) = \frac{Vsg \, \omega^2 Ro \, t}{G} - \frac{Vs}{\omega} \sin(\omega t)$$

$$\text{for } 0 < t < \frac{2\pi}{\omega}$$

(19)

The first term of equation (19) may be physically interpreted as the centrifugally induced radial deviation per revolution. The second term is the radial deviation induced by the radial component of gravity. Both terms in equation (19) are nullified if operated in actual microgravity without rotation. Note that the radial deviation, $Dr(t)$, is at a maximum "negative" number at $t = \pi/2\omega$ (90°), zero at $t = \pi/\omega$ (180°), maximum positive at $t =$

$3\pi/2\omega$ (270°), and "almost" back to zero at $t = 2\pi/2\omega$ (360°). The particle does not return exactly to its original radius because of the centrifugal force which always acts radially outward. The first term of equation (19) is the amount of this centrifugal component of radial deviation which accumulates over time and eventually all particles (which settle) will spiral to the outside wall of a rotating vessel if no other disturbance occurs. The rate of this outward spiral increases with increasing particle radius, vessel rotation rate, and increasing unit gravity particle sedimentation rate, Vsg.

The radial deviations from the circular streamlines, indicated by equation (19) and described above, show that the particle path is nearly the path shown (dotted line) in figure 1.

Tangential Deviation Calculation

The particle also deviates from its original position tangentially along the streamlines. We will find that the particle advances in phase during the first half revolution and retards nearly back to its original phase in the second half revolution. Coriolus acceleration will cause the particle to "lag" the fluid in phase angle as the particle spirals outward due to centrifugal-induced motion.

Equation (16), which follows, is integrated over time to derive the tangential deviation of a particle along a circular streamline. Note that this equation is parametric in that equation (15) is substituted for Vr.

$$D t(t) = \int_0^t Vt(t)\,dt = Vs \int_0^t \sin(\omega t)\,dt - Vsg\,\frac{2\omega}{G}\left[\int_0^t Vr(t)\,dt\right]$$

(20)

Note that equation (19) may be substituted for the bracketed term in equation (20) if we impose the single revolution restriction.

$$D t(t) = \frac{Vs}{\omega}\Bigg|_0^t - \cos(\omega t) - \frac{2Vsg\,\omega}{G}\left[\frac{Vsg\,\omega^2 Ro\,t}{G} - \frac{Vs}{\omega}\sin(\omega t)\right]$$

(21)

$$D t(t) = \frac{Vs}{\omega}[1 - \cos(\omega t)] - \frac{2Vsg^2\omega^3 Ro\,t}{G^2}\,\frac{2Vs\,Vsg}{G}\sin(\omega t)$$

$$\text{for } 0 < t < \frac{2\pi}{\omega}$$

(22)

Equation (22) has the following physical interpretation. The first term represents the gravity-induced advancement and retardation of the particle position along the streamlines. The third term is the coriolus-induced tangential motion due to the radial component of gravity causing radial deviations from the streamlines. The second term is purely due to rotation, not gravity, and is the coriolus-induced tangential motion due to centrifugally induced radial motion. All three terms are nullified if operated in actual microgravity without rotation.

Graph 1 shows the relative importance of the gravity-induced radial displacements and the centrifugally induced radial displacements for the NASA-designed rotating culture vessels. The magnitudes of the radial displacements from each effect are plotted from equation (19).

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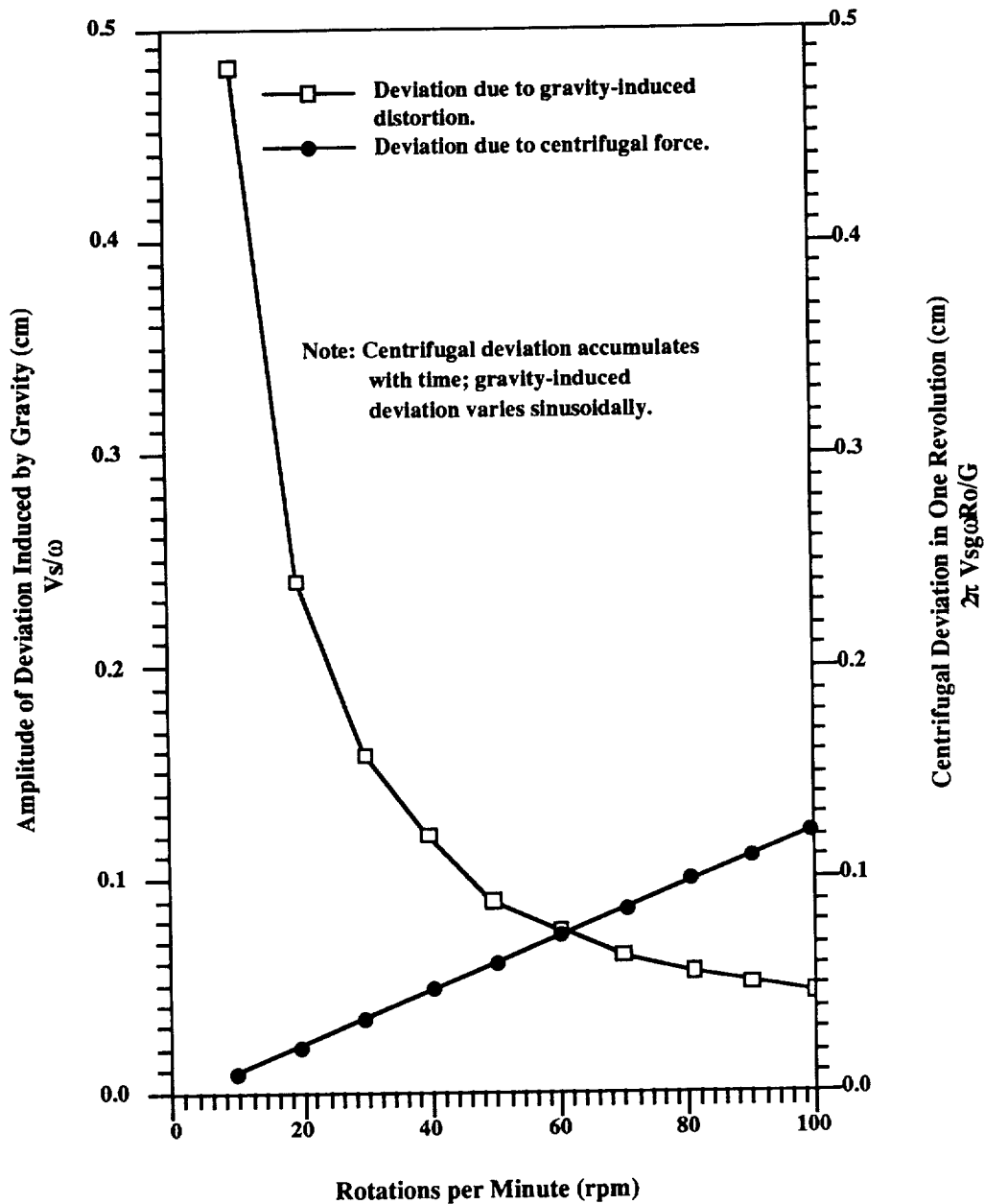
Graph 1. Magnitude of Deviation of Particles Across Streamlines
NASA Rotating Culture Vessel

NASA rotating vessel typical parameters:

RPM - 0 to 100

radius - 3.8 centimeters

G - 980 centimeters per second squared

Vs - 0.5 centimeters per second   (measured Stokes terminal velocity for a typical cultured tissue segment)

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As expected, at low RPM the gravity effects dominate and at high RPM the centrifugal effects dominate. The centrifugal deviation accumulates over time and, thus, is less tolerable than deviation resulting from gravity-induced distortion which is sinusoidal over time. Optimal for these conditions is 40 to 60 RPM. Note that both deviations are nullified by operation in actual microgravity without rotation.

V.   Perfusion Speed of Fluid Through the Culture Vessel

Culture media must be perfused either continuously or intermittently through the culture vessel in order to supply nutrients and clear waste products for long-term culture viability. Perfusion may also be utilized to supply oxygen and to remove carbon dioxide, but this may also be easily accomplished by in-vessel exchange membranes, as our laboratories have demonstrated. It should be noted that such perfusion may be stopped for periods of time sufficient for biological experimentation without adverse effects on the culture. Therefore, we may reduce the fluid flows to essentially zero in microgravity for significant periods of time.

A flow rate of 5.0 milliliters per minute is quite sufficient for nutrient delivery and waste product clearance of a 500-milliliter culture for indefinite periods of time. Figure 3 depicts an assumed typical surface across which this fluid flux crosses.

Vessel Diameter, 8 cm

Spin Filter Diameter, 2.5 cm

Flow Surface Radius
$R_s = 1.5$ cm, $L_s = 8.8$ cm
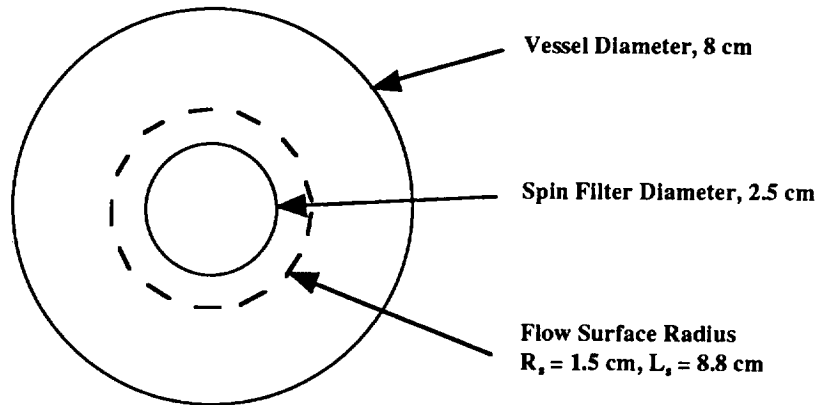
Cross Section of NASA Vessel

Figure 3. Average Flow Field Calculation

The surface is a cylinder of radius 1.5 centimeters and length 8.8 centimeters placed concentrically in the culture vessel. The vessel is designed such that the nutrient media exists through a spin filter distributed within this assumed typical surface. The average fluid speed, Sp, across this surface is calculated by dividing the flow rate by the surface area as follows.
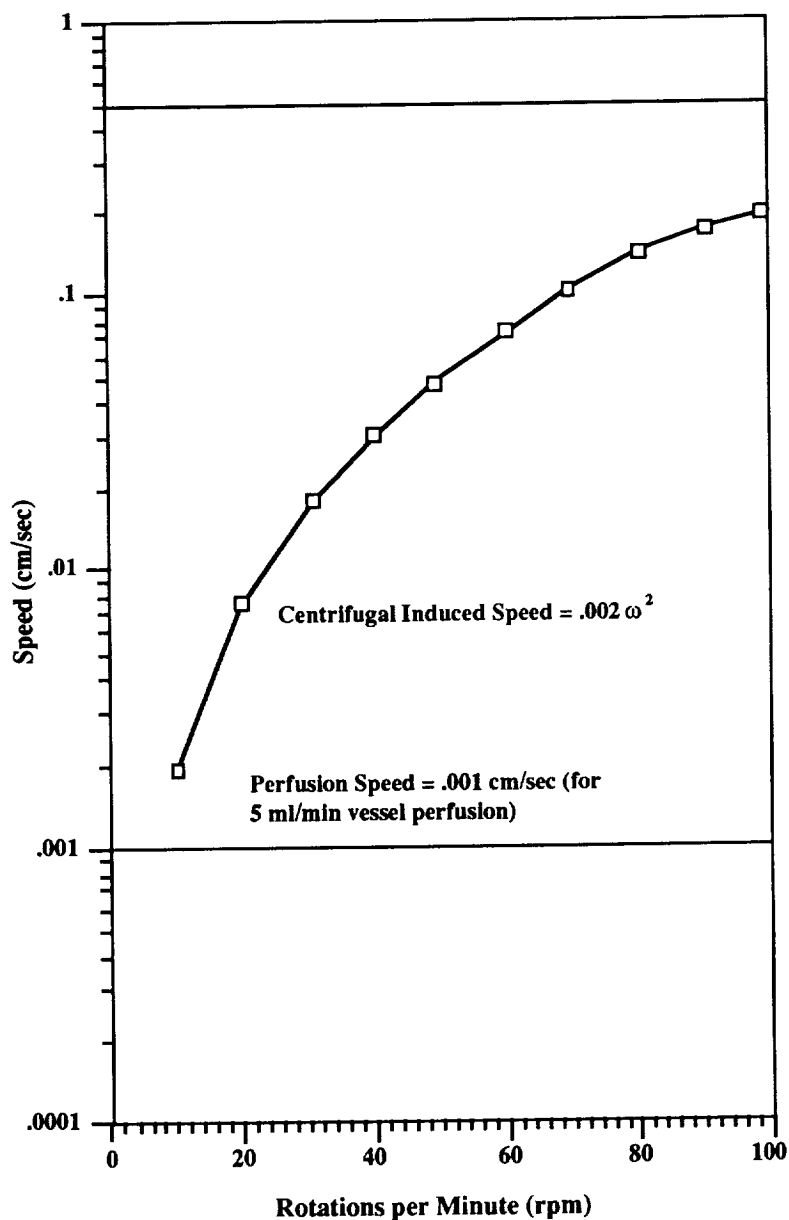
$$Sp = \frac{Flow}{Area} = \frac{Flow}{2\pi R_s L_s}$$

(23)

Five milliliters per minute is equal to 0.0833 milliliters per second. Substituting this with the radius, R_s, and length, L_s, of the surface gives the result, equation (24).

$$Sp = \frac{5\ cc}{min} \bullet \frac{1\ min}{60\ sec} \bullet \frac{1}{2\pi(1.5\ cm)(8.8\ cm)} = \frac{0.001\ cm}{sec}$$

(24)

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The average speed of flow across this surface, under the conditions defined above, is 0.001 centimeters per second. Note that even this small flow field may be essentially eliminated for significant periods of time by intermittent perfusion of the culture vessel in either unit gravity or microgravity. The flows, resulting from particle motion induced by gravity and vessel rotation, are necessarily continuously present in unit gravity and may be completely eliminated in microgravity. Note also that particles with different sedimentation rates assume different orbits in rotating vessels operated in unit gravity.

Graph 2 shows the average flow speed at the surface of the typical tissue segment due to gravitational sedimentation (Stokes velocity), centrifugal-force-induced outward spiral, and fluid media perfusion. It is surprising that in the reference frame of the rotating fluid the center of mass of a particle is actually moving at the Stokes terminal velocity around the circumference of a circular path distorted by centrifugal and coriolus

Centrifugal Induced Speed = $.002\,\omega^2$

Perfusion Speed = .001 cm/sec (for
5 ml/min vessel perfusion)

Speed (cm/sec)

Rotations per Minute (rpm)

Graph 2. Magnitude of Particle Velocity Across Streamlines
NASA Rotating Culture Vessel

effects. For the typical tissue segment chosen in this analysis, the centrifugal and coriolus effects are less than the sedimentation effects, but are significant. We do observe "centrifugation" and "phase lag" of tissues cultured in rotating vessels at rotation rates necessary for adequate suspension. Equations (19) and (22) explain this observed effect.

Figure 4 shows the approximate path of a particle as observed from the rotating reference frame. The particle motion is primarily driven by gravitational acceleration but also significantly influenced by centrifugal and coriolus effects. The particle speed is not constant as it traverses this path because gravity-, centrifugation-, and coriolus-induced motions reinforce and cancel depending on the instantaneous position of the particle.
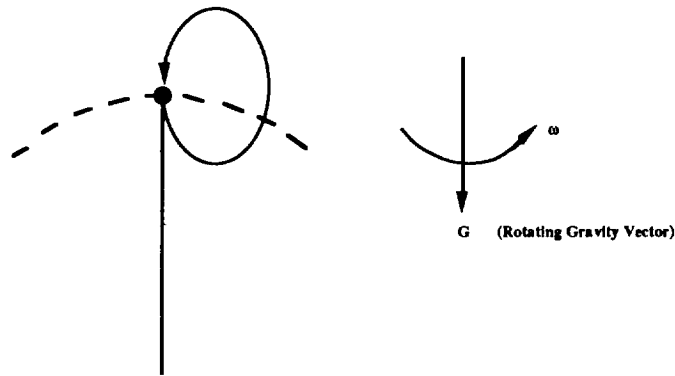

Figure 4. Approximate Path of a Particle

We achieve suspension because the particles return nearly to their original position each vessel revolution. This circular motion must certainly introduce complex secondary motions on the particle and to the fluid. Figure 5 depicts a possible secondary motion of the particle and fluid environment. Such phenomena are complex and difficult to characterize. Completely satisfactory theoretical models and testing methodology which link the large scale bulk flows to the smaller scale secondary effects do not exist (Mixing of Liquids by Mechanical Agitation, Ulbrecht and Patterson, 1985).
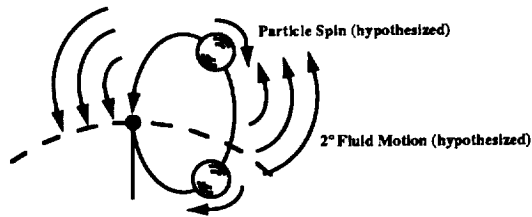

Figure 5. Possible Particle Motion and Secondary Effect

VI.   Conclusions

1.   Typical cultured tissues attain 98% of their terminal velocity (Stokes velocity) in approximately 1 millisecond.

2.   Typical cultured tissues travel at essentially terminal velocity in a circular path distorted by centrifugal and coriolus-induced motions, as observed from the rotating reference frame of the culture vessel (and fluid medium). This motion is primarily driven by the rotating gravity vector. The radius of these "small circles" may be on the order of the tissue radius for high rotational rates.

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3. The NASA rotating culture vessels operate well between 10 and 70 RPM for typical tissues. At lower angular rates the radius of the "small circles" is large and tissues settle to the bottom wall. At higher rates centrifugal effects accumulate rapidly and tissues accumulate at the outer walls.

4. Operation in microgravity will allow a 500 time reduction in the average surface flow across a typical cultured tissue segment, as compared to operation in unit gravity. Intermittent perfusion will allow time periods of approximately 8 hours when this reduction is on the order of 50,000 times.

5. The amount of reduction stated in conclusion 4 will increase with larger cultured tissues which differ more from culture media than the typical tissue segment chosen for this analysis. The amount of reduction obtained for smaller particles, such as individual cells which are very similar to culture media, is expected to be less.

6. Characterization of the fluid dynamic environment of cultured tissue is greatly simplified in microgravity. Characterization of the secondary effects of gravity and rotationally induced particle motions and those of conventional agitation or mixing are highly theoretical and difficult to test.

7. The rotating culture vessels achieve high biological performance primarily because of the absence of a fluid velocity gradient through the boundary layer at the vessel wall. In this respect microgravity is simulated. The rotating vessels do not simulate the bulk fluid dynamic environment to which typical tissues will be exposed when operated in microgravity.

At first it may seem that this analysis leaves us with a conflict as to the observed extremely high tissue viability over conventional culture systems and the calculation that tissues are actually travelling at their terminal Stokes velocities (similar to conventional systems). This may be resolved by addressing the boundary conditions at the vessel walls. The rotating culture system, in unit gravity, effectively eliminates the fluid velocity gradient through this wall boundary layer. The cultured particles essentially "fall" in small circles (as viewed in the rotating reference frame) and are not exposed at any position to high velocity gradients. We have a nearly ideal fluidized bed culture system. It is our interpretation of the available data and analyses that it is this boundary condition that such vessels simulate microgravity.

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